



# JOURNAL OF AGRICULTURAL RESEARCH

## DEPARTMENT OF AGRICULTURE

VOL. VI

WASHINGTON, D. C., MAY 15, 1916

No. 7

### EFFECT OF COLD-STORAGE TEMPERATURES UPON THE PUPE OF THE MEDITERRANEAN FRUIT FLY<sup>1</sup>

By E. A. BACK, *Entomologist*, and C. E. PEMBERTON, *Scientific Assistant, Mediterranean and other Fruit-Fly Investigations, Bureau of Entomology*

#### INTRODUCTION

The use to which cold-storage temperatures may be put as an aid in offsetting the disastrous results of attack by the Mediterranean fruit fly, *Ceratitis capitata* Wied., has already been made the subject of discussion by the writers.<sup>2</sup> In their paper, however, data on the effect of various ranges of temperatures used in commercial cold-storage plants upon the eggs and larval instars only are given. So far as the writers have been able to determine, fruits of almost any variety commonly held in storage are held at temperatures varying from 32° to 45° F., with preference shown to a range of 32° to 36°. The effect upon over 26,000 eggs and 60,000 larvæ of different temperatures, including 32°, 32° to 33°, 33° to 34°, 34° to 36°, 36° to 40°, 38° to 40°, and 40° to 45°, indicate that no eggs or larvæ survive refrigeration for seven weeks at 40° to 45°, for three weeks at 33° to 40°, or for two weeks at 32° to 33°.

While the greatest danger in the spread of this pest from one country to another lies in the transportation of the larvæ within fruits, there are certain data on record which prove that this pest may be carried long distances in the pupal stage and arrive at its destination in a condition to produce infestation. A fruit-fly pupa (species unknown) was found at Auckland, New Zealand, in soil about the roots of plants imported from Australia.<sup>3</sup> In 1914, Sasscer<sup>4</sup> records the discovery in Washington, D. C., of living pupæ of the papaya fruit fly (*Toxotrypana curvicauda* Gerst.) in a package containing an unknown vine from Mexico. In

<sup>1</sup> The writers wish to acknowledge the assistance given them by Mr. H. F. Willard in obtaining the data recorded in this and in their previous paper. To obtain these data has necessitated much prolonged tedious work extending over three years. In securing the data during 1915, Mr. Willard has not only greatly assisted, but on several occasions during the absence of the writers has been entirely responsible not only for the completion of experiments already started, but for the starting of others.

<sup>2</sup> Back, E. A., and Pemberton, C. E. Effect of cold-storage temperatures upon the Mediterranean fruit fly. *In Jour. Agr. Research*, v. 5, no. 15, p. 657-666. 1916.

<sup>3</sup> Kirk, T. W. Fruit flies. New Zeal. Dept. Agr. Div. Biol. Bul. 22, p. 9. 1909.

<sup>4</sup> Sasscer, E. R. Important insect pests collected on imported nursery stock in 1914. *In Jour. Econ. Ent.*, v. 5, no. 2, p. 268-270. 1915.

another instance the same investigator records finding a living adult of the olive fruit fly (*Dacus oleae* Rossi) and a dead adult of another species of fruit fly, apparently *Dacus semisphaerens* Becker. Both of these species were in a small package containing olive seed from Cape Town, South Africa, after having been en route 28 days. Sasser states that according to Silvestri it requires from 47 to 49 days in Italy for the pupæ of the olive fruit fly to yield adults; hence, it is possible for this ruinous pest to enter the United States through the eastern ports as pupæ and reach the olive-growing sections of California before adults have emerged.

Such facts as these indicate that the Mediterranean fruit fly may be similarly transported, and emphasize the desirability of recorded data on the effect of cold-storage temperatures upon the pupal stages. Aside from the practical application in the future to quarantines regulating the shipment of fruits, the results given below throw considerable light on conditions governing the distribution of the pest, and help explain the varying severity of its ravages in countries having both semitropical and temperate fruit-growing regions.

#### HISTORICAL REVIEW

Practically nothing has been published on the effect of cold-storage temperatures upon the pupæ of *Ceratitis capitata*. In 1908 Lounsbury<sup>1</sup> in South Africa reports that in removing fruit infested with *C. capitata* from refrigeration at 38° to 40° F. at the end of 21 and 27 days he found in each instance a single pupa, but that both proved to be dead. The experiments of the writers have demonstrated that these two pupæ were produced by larvæ which formed their puparia before the fruit was placed in storage, as larvæ do not form puparia at temperatures lower than 45° to 48° F.

In 1914 Newman,<sup>2</sup> in Western Australia, placed one box containing 50 newly formed puparia in each of four rooms held, respectively, at 32°, 36°, 45°, and 55° F. At the end of 34 days of refrigeration 25 pupæ were taken from each box held at 32° and 36°, and at the end of 70 days of refrigeration the remaining pupæ held at 32° and 36° and all held at 45° and 55° were removed to the laboratory. None of the pupæ removed yielded adults.

#### EXPERIMENTAL WORK

Nearly all the experimental work with temperatures lower than 45° F. was carried on in a thoroughly modern three-story cold-storage plant. The temperatures of the rooms in this plant were held quite definitely within certain fixed ranges by hourly inspections made by the storage employees. One experiment was carried on in a second plant where, as indicated in the text, the temperature was subject to considerable fluctuation.

<sup>1</sup> Lounsbury, C. P. Report of the Government Entomologist, Cape of Good Hope, 1907, p. 56. 1908.

<sup>2</sup> Newman, L. J. Annual report of the officer in charge of the insectary for the year ended June 30, 1914. *Jn Ann. Rpt. Dept. Agr. West. Aust.* 1914, p. 61. 1915.

tuation. The temperatures 49° to 51°, 52° to 56°, and 54° to 57° were not obtainable in the Honolulu cold-storage plants, hence in experiments at these temperatures ordinary refrigerators were used, as indicated. Usually pupæ of all ages from 1 to 9 or 10 days were obtained for each experiment, in order that varying effects upon pupæ in different stages of development might be noted. The pupæ were sifted from sand beneath host fruits and placed in storage either in bulk of several thousand in large jars or, as was more usual, in smaller lots of from one to several hundreds in vials about 1 inch in diameter and stoppered with cotton. Pupæ were not placed in or on damp sand or soil, as early experimental work indicated no advantage from this treatment when pupæ are subjected to cold-storage temperatures. The humidity of the storage rooms varied between 80° and 91°. After refrigeration the pupæ were removed to the laboratory, where they were daily observed for emergence records.

The term "pupa" is used to designate that period in the life history between the formation of the puparium by the larva and the emergence of the adult.

TEMPERATURE, 32° F.—Of the 13,900 pupæ of all ages subjected to refrigeration at a temperature varying less than half a degree either above or below 32° F. during the experiment, none survived more than 10 days. In Table I are recorded the results of observations on pupæ refrigerated from 2 to 10 days.

TABLE I.—*Effect upon Mediterranean fruit-fly pupæ of refrigeration at 32° F. for from 2 to 10 days*

Age of pupæ on entering storage	Number of pupæ yielding adults after removal to normal temperature after refrigeration for—									
	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.	10 days.	
1 day.....	15	6	3	0	1	0	0	0	0	
2 days.....	20	28	20	17	15	4	0	0	0	
3 days.....	32	21	22	10	4	4	3	1	0	
4 days.....	18	17	11	9	1	0	0	0	0	
5 days.....	28	18	3	1	1	1	0	0	0	
6 days.....	29	27	16	6	1	0	0	0	0	
7 days.....	48	33	13	5	2	4	0	0	0	
8 days.....	52	39	17	7	2	0	0	0	0	
9 days.....	51	33	21	12	4	0	0	0	0	

Each lot removed after from 2 to 8 days of refrigeration contained 100 pupæ; hence, the number of pupæ yielding adults represents also the percentage of survival. Very few pupæ survived refrigeration at this temperature for longer than one week. Thus only 3 three-day-old pupæ out of 900 pupæ of all ages survived refrigeration for 8 days, and only 1 three-day-old pupa survived refrigeration for 9 days. While the data in Table I do not show it, the one surviving 9 days of refrigeration was one out of 300 of like age, and one out of 1,900 of all ages. Not one of 4,500 pupæ refrigerated for 10 days survived.

TEMPERATURES OF FROM 33° TO 34° F.—Only 3 out of 207 pupæ held at 33° to 34° F. for 4 days and at 43° to 45° F. for 8 additional days yielded adults.

TEMPERATURES OF FROM 33° TO 36° F., AVERAGING 34°.—A total of over 27,097 pupæ were used in experiments to determine the effect of a temperature averaging about 34° F. but varying between 33° and 36° F. Only 1 seven-day-old pupa out of 228 of like age or 1,239 of all ages refrigerated for 16 days yielded an adult; 1,228, 1,164, 1,694, and 1,931 refrigerated for 18, 20, 22, and 25 days were dead on removal from storage. Only 3 out of 272 seven-day-old pupæ, or 1,472 pupæ of all ages, produced adults after refrigeration for 15 days, while only 8 out of 210 eight-day-old pupæ and 3 out of 220 seven-day-old pupæ, or but 11 out of 1,630 pupæ of all ages from one to eight days old when placed in storage, produced adults after refrigeration for 14 days. After refrigeration for 12 days, 12 eight-day-old pupæ, 11 seven-day-old pupæ, 2 six-day-old pupæ, and 8 one-day-old pupæ out of a total of 1,580 pupæ of all ages produced adults. From 1 to 30 adults emerged from lots of all ages of pupæ, totaling 1,519 forms, except from 126 five-day-old pupæ, after refrigeration for 11 days, but from 1 to 3 adults emerged from all lots yielding adults, except from the seven-day-old pupæ, which yielded 30 adults from a total of 265 pupæ.

Refrigeration of 1,685 pupæ of all ages for 9 days did not prove totally fatal to any age. Thus 85 out of 340 eight-day-old pupæ, and 88 out of 390 seven-day-old pupæ produced adults as compared with 3 four-day-old pupæ, 7 three-day-old pupæ, and 2 one-day-old pupæ out of a total of 475 pupæ.

Some adults emerged from lots of pupæ representing all ages on removal from storage after 2, 3, 4, 5, 6, 7, and 8 days of refrigeration. On these days an average of about 1,479 pupæ were removed from storage. The number of pupæ surviving is indicated by the data in Table II.

TABLE II.—*Effect upon pupæ of the Mediterranean fruit fly of refrigeration for from 1 to 8 days at 33° to 36° F.*

Age of pupæ on entering storage.	Number of pupæ yielding adults after removal to normal temperature after refrigeration for—						
	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.
1 day.....	87	59	56	59	16	15	9
2 days.....	41	12	25	6	8	2	6
3 days.....	49	76	44	18	12	14	6
4 days.....	27	47	26	11	4	5	3
5 days.....	12	21	14	10	10	1	4
6 days.....	10	15	17	10	5	8	2
7 days.....	190	405	228	221	188		98
8 days.....	129	153	434	146	216	150	91

The data in Table II are introduced to prove that refrigeration for from 1 to 8 days at this temperature is not fatal, and can not be depended

upon to kill all pupæ. While an average of about 1,479 pupæ were removed each day, the number of pupæ of each age is not known; hence no conclusion can be drawn regarding the relative effect of this refrigeration upon pupæ of different ages. The data given above for 9, 11, 12, 14, 15, and 16 days of refrigeration seem to indicate that the older pupæ withstand the effects of cold for a relatively longer period.

In a second experiment 50 out of 200 pupæ of all ages yielded adults after 4 days' refrigeration, and but 15 out of 207 pupæ held at 33° to 34° F. for 4 days and then at 43° to 45° F. for 3 additional days.

TEMPERATURES OF FROM 28° TO 40° F., AVERAGING 36°.—A total of 8,500 pupæ were placed in a cold-storage room the temperature of which was subject to far greater changes than are usual in commercial plants. While the temperature averaged about 36° F. a large portion of the time, for short periods during the night it dropped to freezing or even 28°, and during the heat of the day when supplies were being removed frequently rose to 38° to 40°. As each lot of the various ages from ½ to 9 days removed consisted of 100 pupæ, the numbers of pupæ yielding adults after the various numbers of days of refrigeration represent the percentages of survival. In Table III are recorded the effects of from 1 to 24 days of refrigeration on 6,800 pupæ.

TABLE III.—*Effect upon pupæ of refrigeration at temperatures varying between 28° and 40° F., but averaging about 36° F.*

Age of pupae on entering storage.	Number of pupae yielding adults on removal to normal temperature after refrigeration for—								
	1 day.	3 days.	6 days.	8 days.	10 days.	12 days.	15 days.	18 days.	24 days.
½ day.....	81	2	0		0	0	0		
1 day.....	92	35	0		0			0	
2 days.....	62	5	0	0	0	0	0	0	0
3 days.....	81	2	0	0	0	0	0	0	0
4 days.....	55	3	0	0	0	0	0	0	0
5 days.....	70	32	14	7	0	0	0	0	0
6 days.....	94	21	10	0	0	0	0	0	0
7 days.....	69	60	51	10	1	0	0	0	0
9 days.....	" 31	" 27	" 23	25	0	0			

0 of these three lots of 100 pupæ each, 25, 17, and 10 pupæ, respectively, yielded adults July 8, or just before being placed in cold storage.

A total of 1,700 pupæ of various ages removed to normal temperature after refrigeration for 21, 27, 29, and 31 days were found to be dead.

TEMPERATURES OF FROM 38° TO 40° F.—A total of 52,604 pupæ were used in experiments to determine the effect upon pupæ of refrigeration at 38° to 40° F. An average of 1,860 pupæ of all ages were removed after refrigeration for 3, 4, 6, 7, 8, 10, 12, and 14 days. The number of pupæ for each age varied from 109 to 414 and averaged 234. The number of pupæ surviving refrigeration for from 3 to 14 days is recorded in Table IV.

TABLE IV.—Effect upon Mediterranean fruit-fly pupæ of refrigeration for from 3 to 14 days at 38° to 40° F.

Age of pupæ on entering storage.	Number of pupæ yielding adults on removal to normal temperature after refrigeration for—							
	3 days.	4 days.	6 days.	7 days.	8 days.	10 days.	12 days.	14 days.
1 day.....	110	13	5	1	5	1	0	1
2 days.....	150	170	75	110	121	145	124	42
3 days.....	62	58	81	23	4	6	4	4
4 days.....	35	41	16	13	10	4	1	1
5 days.....	91	86	52	63	24	9	12	6
6 days.....	132	82	59	52	23	6	7	0
7 days.....	235	117	114	121	96	60	32	22
8 days.....	207	234	136	161	180	61	63	19

After refrigeration for 17 days only 3 out of 306 eight-day-old pupæ, 3 out of 384 seven-day-old pupæ, 3 out of 206 six-day-old pupæ, 1 out of 162 four-day-old pupæ, and 11 out of 374 two-day-old pupæ yielded adults, or only 21 out of 2,352 pupæ of all ages survived.

After refrigeration for 18 days only 9 out of 701 eight-day-old pupæ, 5 out of 250 seven-day-old pupæ, 1 out of 295 five-day-old pupæ, 1 out of 430 three-day-old pupæ, and 13 out of 400 two-day-old pupæ yielded adults; or only 29 out of 2,632 pupæ of all ages survived.

Nineteen days of refrigeration proved fatal to 1,911 pupæ of all ages except 2 out of 375 one-day-old pupæ. No living pupæ were found among 2,031 pupæ of all ages after refrigeration for 21 days, nor among 28,700 pupæ of all ages after refrigeration for 35 days.

TEMPERATURES OF FROM 40° TO 45° F.—In this experiment to determine the effect upon pupæ of temperatures ranging between 40° and 45° F., 8,800 pupæ from 1 to 10 days old were used. Each unit of pupæ contained 100 forms; hence, the numbers of pupæ yielding adults after refrigeration from 1 to 27 days as recorded in Table V represent the percentages of survival.

TABLE V.—Effect upon Mediterranean fruit-fly pupæ of refrigeration at 40° to 45° F. for from 1 to 27 days

Age of pupæ on entering storage.	Number of pupæ yielding adults after removal to normal temperature after—											
	1 day.	3 days.	6 days.	8 days.	10 days.	12 days.	16 days.	18 days.	24 days.	27 days.		
1 day.....	22	78	15	3	1	13						
2 days.....	81	77	59		24			3				
3 days.....	69	91	1	1	2	0		0	0	0		
5 days.....	47	63	38	13	20	13	0	0	0	0	0	
6 days.....	66	82	72	58	49	31	5	0	0	0	0	
7 days.....	91	87	75		60	57						
8 days.....	55	60	44	54	27	16	4	1	1	1	1	
9 days.....												
10 days.....	<sup>a</sup> 17	<sup>a</sup> 23	<sup>a</sup> 21	8	1	<sup>a</sup> 1		0				

<sup>a</sup> Besides these, 13, 28, 30, and 10 pupæ, respectively, yielded adults just before pupæ were placed in cold storage.

It will be noted that only 9 out of 300 pupæ survived refrigeration for 16 days, while only 4 out of 500 and 1 out of 500 refrigerated for 18 and 24 days, respectively, survived. Three hundred pupæ refrigerated for 31 days and 200 refrigerated for 34 days were found dead on removal.

TEMPERATURES OF FROM 49° TO 51° F.—Temperatures ranging between 49° and 51° F. and averaging about 50° have proved most interesting of all, as these appear to be very close to the point below which the insect's activities cease. This temperature was secured by use of an ordinary refrigerator 42 by 34 by 18 inches. During the period from May to July, 1914, 31,700 pupæ were used in an experiment to determine the effect of this temperature upon pupal development. Pupæ in 15 lots, of ages ranging from 1 to 8 days, and averaging 3,523 pupæ for each of the 8 days represented, were held in storage for two months before removal. Frequent observations were made but no pupæ completed their development and yielded adults in storage. On removal to normal temperature all of the 31,700 pupæ were found dead.

The second lot of 7,800 pupæ placed in storage when 5 days old yielded a few adults. Thus, 9 out of 7,800 yielded 1, 2, 2, 3, and 1 adult in storage after refrigeration for 20, 23, 44, 46, and 47 days. In other words, it took these 9 pupæ from 20 to 47 days to accomplish the development in refrigeration which at an outdoor temperature at that season, July, 1914, would have taken only from 4 to 5 days.

TEMPERATURES OF FROM 52° TO 56° F.—Ten larvæ pupating in a refrigerator held at 52° to 56° F. yielded 2 and 1 adult in storage after refrigeration for 38 and 52 days, respectively. The remaining 7 pupæ died.

TEMPERATURES OF FROM 54° TO 57° F.—Temperatures of from 54° to 57° F. were obtained by using an ordinary refrigerator 46 by 27 by 18 inches. A total of 22,700 pupæ were used varying in age from  $\frac{1}{2}$  to 9 days. Not less than 1,400 pupæ, or more than 3,500 pupæ of any age, were used. In Table VI are recorded the reactions of 3,100 one-day-old pupæ to these temperatures.

From the data in Table VI it will be noted that 54° to 57° F. is not in all cases fatal to pupal development, although a high mortality occurs. Each outward date represents 100 pupæ. As the heavy line extending diagonally across the table indicates the dates on which pupæ were removed from refrigeration, and as the normal pupal development is completed at this season of the year at Honolulu in from 9 to 12 days, the data prove that development continues at this temperature as evidenced, first, by the rate of emergence of adults after the pupæ are removed from refrigeration up to the thirtieth day of refrigeration, and, secondly, by the emergence actually occurring within storage on the thirty-first day and up to the thirty-seventh day of refrigeration. Thus development was wholly completed and emergence had taken place at this temperature among pupæ removed from refrigeration after 37, 38, and 39 days.



TABLE VI.—Effect upon 1-day-old Mediterranean fruit-fly pupæ of refrigeration at 54° to 57° F. Pupæ placed in refrigeration August 22, 1913

		Date and emergence of adults.																																
		August.										September.																						
Outward date.		28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Aug.	24.....	1	0	0	10	3	3																											
	26.....	1	0	2	4	25	21																											
Sept.	28.....													27	19	1																		
	3.....													29	36																			
	4.....													1	0	0	15	14	1															
	5.....																																	
	6.....																																	
	7.....																																	
	8.....																																	
	9.....																																	
	10.....																																	
	11.....																																	
	12.....																																	
	13.....																																	
	14.....																																	
	15.....																																	
	16.....																																	
	17.....																																	
18.....																																		
19.....																																		
20.....																																		
21.....																																		
22.....																																		
23.....																																		
24.....																																		
25.....																																		
26.....																																		
27.....																																		
28.....																																		
29.....																																		
30.....																																		

Of 1,700 pupæ one-half day old when placed in refrigeration none emerged within storage until the twenty-fourth day of refrigeration, after which no data were secured. One hundred pupæ removed after 24 days of refrigeration produced 2, 27, and 5 adults 4, 5, and 6 days after removal, proving that even among these very young pupæ development was not completely arrested. Out of two lots of 100 pupæ each, 46 and 44 pupæ, respectively, refrigerated for 22 and 23 days, yielded adults 3, 4, and 5 days after removal from storage.

Data on file bring out an interesting fact that might be expected from the data in Tables VI and VII. The older the pupæ when placed in refrigeration, the quicker they develop and produce adults while in refrigeration. Thus in Table VII are recorded data on the development of six-day-old pupæ.

It will be noted that while a few one-day-old pupæ require a minimum of 31 days of refrigeration for development, 2 six-day-old pupæ com-

pleted their development and produced adults in storage on the fifth day of refrigeration, and that thereafter emergence of adults continued until all living pupæ yielded adults in storage by the end of the sixteenth day of refrigeration except 2, which yielded adults on the fourth day after removal after 16 days of storage. Data on 1,900 eight-day-old pupæ show that from 1 to 5 pupæ among each of 14 different lots of 100 completed their development and yielded adults on the second day of refrigeration, that an average of 43.5 per cent of 14 lots yielded adults on the third day, and that emergence of adults was completed by the seventh day except in one instance where 2 pupæ yielded adults in storage on the ninth and tenth days of refrigeration.

TABLE VII.—*The effect upon six-day-old Mediterranean fruit-fly pupæ of refrigeration at 54° to 57° F. Pupæ placed in refrigeration August 22, 1913*

Outward date.	Date and emergence of adults.																			
	August—										September—									
	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10			
Aug. 24.....			57	6																
26.....			1	45	29				1											
27.....				2	37	0	22	0	1											
28.....						4	67													
Sept. 3.....						1	0	10	8	19	13	0	3							
4.....							13	13	13	7	11									
5.....			0	0	0	0	13	13	12	12	12	2	3							
6.....				1	1	0	7	12	16	15	17	10	4							
7.....				1	0	1	0	11	10	13	9	19	7	2	0	0	0	2		
8.....				1	1	0	0	13	10	13	13	11								
9.....					2	2	0	11	5	9	15	10	2							
10.....					1	0	0	13	21	13	9	18	3	2						
12.....				0	0	0		11	6	13	10	13	1	2	3					

Data on file covering observations on 1,600, 1,400, 1,700, 2,200, 3,300, 4,000, 3,100, 3,500, and 1,900 pupæ, 1, 2, 3, 4, 5, 6, 7, 8, and 9 days old, respectively, show a steady increase in the pupæ completing their development and yielding adults in refrigeration, and their tabulation shows a transition from the condition in Table VI through that of Table VII to the condition set forth for eight-day-old pupæ. One lot of two-day-old pupæ left in storage for 37 days yielded adults almost daily between the twenty-sixth and thirty-fifth days of refrigeration. Eight lots of three-day-old pupæ left in storage from 32 to 39 days yielded adults between the twenty-third and thirty-third days of refrigeration and none thereafter. Fourteen lots of four-day-old pupæ in storage from 25 to 39 days yielded adults between the sixteenth and twenty-seventh days of refrigeration and none thereafter. Eight lots of seven-day-old pupæ in storage from 13 to 21 days yielded adults between the second and eleventh days of refrigeration and none thereafter.

## CONCLUSION

From the data secured during experimental work reported on the foregoing pages, including observations on 173,318 pupæ of the Mediterranean fruit fly (*Ceratitis capitata* Wied.), it appears that no pupæ survive refrigeration for longer periods than is necessary to cause the death of eggs and larvæ in host fruits held at corresponding temperatures.

About 50° F. is the critical point below which development can not take place and below which death will follow if refrigeration is continued sufficiently long. At 49° to 51° only 9 out of 39,500 pupæ yielded adults in refrigeration 20 to 47 days after the inward date, while 3 out of 6 held at 52° to 56° yielded adults in refrigeration 38 to 52 days after the inward date. Many pupæ can complete their entire development in refrigeration at 54° to 57°, while higher temperatures, not considered here, merely retard development without causing noticeable mortality.

Pupæ can not withstand temperatures below 50° F. for prolonged periods of time. Only 3 and 1 pupa survived refrigeration for 8 and 9 days, respectively, at 32°, while none of 4,500 pupæ survived 10 days at this temperature. Refrigeration at a temperature averaging 34°, but ranging between 33° and 36°, proved fatal after the seventeenth day; 6,017 pupæ refrigerated at this temperature for 18 and 25 days yielded no adults, while the number to yield adults after refrigeration for 14 and 17 days was very small. No pupæ survived refrigeration at 28° to 40° but averaging 36°, for more than 10 days. A temperature of 38° to 40° proved fatal after the nineteenth day; 30,731 pupæ refrigerated for from 21 to 35 days failed to yield adults on removal to normal temperatures. After refrigeration at 40° to 45° pupæ from each of two lots removed after refrigeration for 24 and 27 days, respectively, yielded adults; 500 pupæ removed after refrigeration for from 31 to 34 days proved to be dead.

It does not seem safe to conclude that the age of the pupa has a direct bearing upon its ability to withstand the more ordinary ranges of cold-storage temperatures.

## EFFECT OF CLIMATIC FACTORS ON THE HYDRO-CYANIC-ACID CONTENT OF SORGHUM

By J. J. WILLAMAN and R. M. WEST, *Assistant Chemists, Agricultural Experiment Station of the University of Minnesota*

[In collaboration with F. S. Harris, *Agronomist, Utah Agricultural Experiment Station*; L. E. Call, *Agronomist, Kansas Agricultural Experiment Station*; and Beyer Anne, *Superintendent, Belle Fourche Experiment Farm, Newell, S. Dak.*]

### INTRODUCTION

The present experiments are a continuation of those carried out in 1914 (10)<sup>1</sup> on sorghum (*Sorghum vulgare*). In the latter a correlation was sought between the soil conditions, especially the supply of nitrogen, and the amount of the cyanogenetic glucosid (dhurrin) in the sorghum. It was found that on fertile soils nitrogenous fertilizer has no appreciable effect, but on poor soil added nitrogen may increase the amount of hydrocyanic acid, though only to a small extent. Since the evidence indicated that climate and variety may be more important factors than soil nitrogen in determining the amount of the glucosid in this plant, experiments were carried out during 1915 to study the effect of climatic conditions. It was thought that conditions of high or low temperature, much or little available water, slow or rapid growth, might affect the metabolism of sorghum sufficiently, not only to show the causes of the varying amount of dhurrin, but also to throw some light on the physiological function of this glucosid.

### EXPERIMENTAL WORK

Seeds of two varieties of sorghum were obtained. One was Early Amber, grown in Minnesota, and is designated in these experiments Variety N. The other was Southern Cane, a variety similar to the first, but grown in Missouri. It is designated Variety S. In order to secure as widely varying climatic conditions as possible, one-twentieth-acre plots of each variety were grown at four different State experiment stations. A brief description of each plot follows:

1. University Farm, St. Paul, Minn. Very fertile, black loam, fair drainage. Planted on June 3; sprouted on June 12; cultivated twice. Season very cold and wet; sorghum three or four weeks behind the normal in development; did not reach maturity, but was killed by frost in the soft dough stage.
2. Agricultural Experiment Station, Logan, Utah. Irrigation farming. Plots on McNiel farm, North Logan; the two varieties alternated with beans; soil a clay loam, rich in manure. Planted May 15; appeared aboveground on June 1; irrigated on July 9 and August 11; cultivated on June 10, June 17, July 1, July 13, and August 17. Rainfall up to June 10 was abnormally high, which kept the soil cold and retarded growth of crops. During the rest of the season optimum moisture content of soils

<sup>1</sup>Reference is made by number to "Literature cited," p. 272.

obtained. Sorghum made slow growth; leaves yellowish for first seven or eight weeks.

3. Agricultural Experiment Station, Manhattan, Kans. Plots grown on "creek bottom land," broken from native sod in 1913; drainage poor. Planted on June 15; appeared aboveground on June 22; cultivated twice. Sorghum 30 days slower in maturing than usual, owing to excessive rains.

4. Belle Fourche Experiment Farm, Newell, S. Dak. Dry farming. Planted on June 10; appeared aboveground on June 26; cultivated on July 23 and August 7; harvested on October 12. Season cold and wet; rainfall far above normal.

5. Belle Fourche Experiment Farm, Newell, S. Dak. Irrigation farming. Planted on May 31; appeared aboveground on June 26; cultivated on July 23 and August 10; irrigated on August 17; harvested on September 16, when plants were just headed out.

From the time when the plants were from 20 to 30 cm. in height, samples were taken every 10 days. They were usually cut between 9 and 12 a. m., although it has been found that the time of day makes no difference in the amount of hydrocyanic acid present. Plants were selected which represented the average of the plot on that date. For the first sample the whole plant was cut into 1-inch lengths and packed into a 600 c. c. friction-top tin can with 20 c. c. of 3 per cent alcoholic sodium hydrate and 2 c. c. chloroform for preservatives and sent to the Minnesota laboratory for analysis. For the other samples the leaves were cut off where they join the sheath, and the leaves and stalks were packed and analyzed separately. The weight of leaves and of stalks in the total sample cut was recorded. From the fourth sample on, cans of 1,600 c. c. capacity were used. An alkaline preservative was used so as to prevent any possible loss of hydrocyanic acid set free by enzymic activity. Alcohol instead of water was used as a solvent for the alkali, because it penetrates the plant tissues more readily. The chloroform prevented any fermentative changes. In the case of the South Dakota, Kansas, and Utah samples, from two to five days elapsed from the time the samples were cut till they were analyzed. In the case of the Minnesota samples, the fresh material was analyzed. In order to test the efficiency of the preservative, several samples from the Minnesota plots, representing the various stages of maturity of the samples outside of Minnesota, were analyzed for hydrocyanic acid before and after storage in cans, with the results given in Table I.

TABLE I.—Efficiency of an alkaline preservative in preventing loss of hydrocyanic acid in sorghum

Preservative treatment.	Percentage of hydrocyanic acid in dry matter.	
	Fresh.	Preserved.
Preserved for four days with alcoholic sodium hydroxid and chloroform.....	0.019	0.020
Do.....	.026	.029
Preserved for eight days with alcoholic sodium hydroxid and chloroform.....	.009	.009
Do.....	.016	.019

The differences noted are within the limits of accuracy of sampling and analyzing; hence, this method of preservation can safely be used on sorghum plants at least through the stages of maturity represented in these experiments.

About 50 gm. of the sample, after thorough mixing and fining with a knife, were used to determine the percentage of dry matter. For the determination of the hydrocyanic-acid content, from 50 to 70 gm. were ground in a food chopper, placed in an 800 c. c. Kjeldahl flask, together with 250 c. c. of 5 per cent tartaric acid, and distilled slowly into 10 c. c. of 2 per cent sodium hydroxid until the distillate was nearly 100 c. c. This completely hydrolyzes the dhurrin and carries the hydrocyanic acid over into the alkaline distillate. The latter was made to 100 c. c. and aliquots used for the determination of hydrocyanic acid according to the method of Viehoefier and Johns (9). This method was found to be easier and more accurate than the thiocyanate method used in 1914.

The complete analytical results appear in Table II. The figures for the amount of hydrocyanic acid in the whole plant were computed from the relative proportion of leaves and stalks in each sample.

TABLE II.—Hydrocyanic-acid content of sorghum from the various experimental plots

(The percentage of hydrocyanic acid is reported on a dry-matter basis)

Plot and sample No.	Date of sampling.	Age of plants since sprouting.	Plot N.				Plot S.			
			Height of plants.	Percentage of hydrocyanic acid.			Height of plants.	Percentage of hydrocyanic acid.		
				Stalks.	Leaves.	Whole plant.		Stalks.	Leaves.	Whole plant.
Minnesota:										
1	July 15	33	25			0.114	25			0.079
2	July 24	42	44	0.026	0.011	0.008	48	0.018	0.015	0.019
3	Aug. 3	52	68	0.018	0.021	0.019	65	0.015	0.021	0.026
4	Aug. 13	62	90	0.012	0.008	0.009	88	0.013	0.011	0.016
5	Aug. 24	71	135	0.009	0.004	0.005	133	Trace.	0.007	0.003
6	Sept. 3	83	160	Trace.	0.004	0.001	186	0.000	0.004	0.001
7	Sept. 13	93	188			Trace.	198		0.002	Trace.
8	Sept. 23	103	208				208		0.003	Do.
Utah:										
1	July 19	49	36			0.034	36			0.040
2	July 29	59	58	0.038	0.013	0.019	59	0.039	0.015	0.031
3	Aug. 7	68	78	0.019	0.013	0.021	84	0.025	0.019	0.029
4	Aug. 18	79	120	Trace.	0.012	0.009	110	0.007	0.014	0.020
5	Aug. 28	89	161	0.000	0.005	0.008	186	Trace.	0.041	0.016
6	Sept. 7	99	174				175			
7	Sept. 17	109	190				192			
Kansas:										
1	July 16	24	18			0.016	26			0.014
2	July 27	15	72	0.000	0.017	0.008	95	0.000	0.030	0.014
3	Aug. 5	44	157	0.000	0.007	0.003	150	0.000	0.020	0.008
4	Aug. 16	55	209				210			
5	Aug. 25	64	206				235			
Dakota (dry farming):										
1	July 26	30	33			0.000	33			0.026
2	Aug. 5	40	61	0.004	0.013	0.011	61	0.009	0.021	0.016
3	Aug. 14	49	91	0.000	0.000	0.000	96	0.000	0.008	0.004
4	Aug. 24	59	144	0.000	0.004	0.002	137	0.000	0.006	0.003
5	Sept. 4	70	186				187			
6	Sept. 14	80	283				287			
Dakota (irrigation):										
1	July 26	30	35			0.009	37			0.026
2	Aug. 5	40	57	0.004	0.008	0.005	51	Trace.	0.009	0.005
3	Aug. 14	49	95	0.000	Trace.	Trace.	91	0.000	Trace.	Trace.
4	Aug. 24	59	134	0.000	do.	do.	134	0.000	0.001	Do.
5	Sept. 4	70	291				290			

In figure 1 the percentages of hydrocyanic acid in the whole plant are plotted against the age in days. No noteworthy differences were noticed when the height of the plants was used instead of the age in days.

Figure 2 represents the growth curve of the various plots, where the height in centimeters is plotted against the age in days since sprouting.

In order to study the relation between climatological factors and the content of hydrocyanic acid, figures 3 and 4 were constructed. In

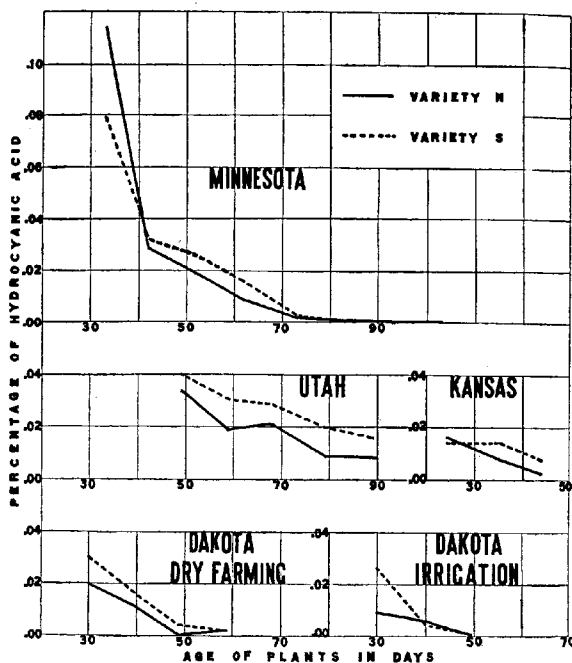


FIG. 1.—Curves showing the hydrocyanic-acid content of sorghum on the various plots. (Percentage of hydrocyanic-acid computed to dry-matter basis.)

figure 3 are plotted first the precipitation (in inches) during 15-day intervals; second, the temperature (degrees Fahrenheit), using averages for 10-day periods, and, third, the mean relative humidity (percentage) at 6 a. m., all for the five months May to September, inclusive. In figure 4 the history of each plot for the season is shown and includes the rainfall, temperature, and hydrocyanic-acid curves on the same graph. The dates for planting, sprouting, appearance of seed panicles, and irrigations are also shown.

## DISCUSSION OF RESULTS

The season of 1915 furnished some excellent extremes in weather conditions for this experiment. Figure 2 shows that, as regards temperature, the two more southern States, Kansas and Utah, form one pair, and South Dakota and Minnesota another, with approximately 10 degrees difference between them during the growing season. Of the two warmer stations, Utah had a low rainfall, and irrigation was resorted

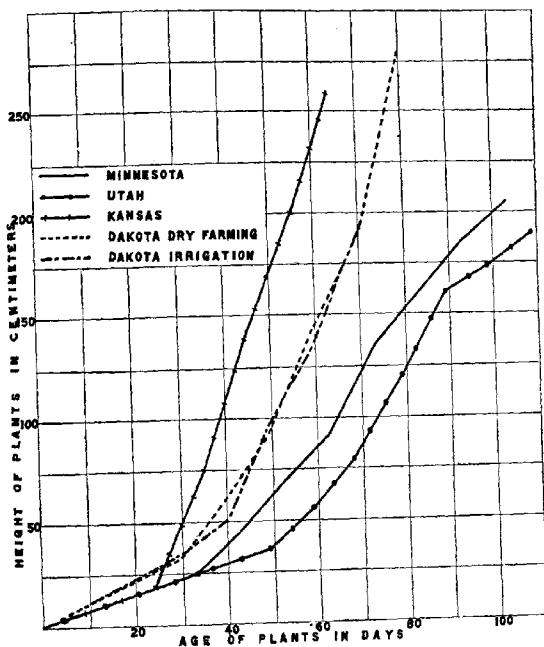


FIG. 2.—Curves showing the rate of growth of the sorghum on the various plots.

to; while Kansas had a very abundant rainfall, resulting even in flood conditions during May, June, and July. The two more northern stations had about the same rainfall for the first three months of the experiment, but during the period when the samples were taken the rainfall of South Dakota dropped below that of Minnesota. This was particularly the case during August. The 1915 rainfall of South Dakota was above normal, and as a result the plot on irrigation ground was irrigated only once.



The five plots differed rather widely in their rate of growth, as is shown in figure 4. The Utah plants were 48 days old before attaining the height required for first sample. During this time they looked yellow and unthrifty, owing to excess moisture and cool soil. Subsequently

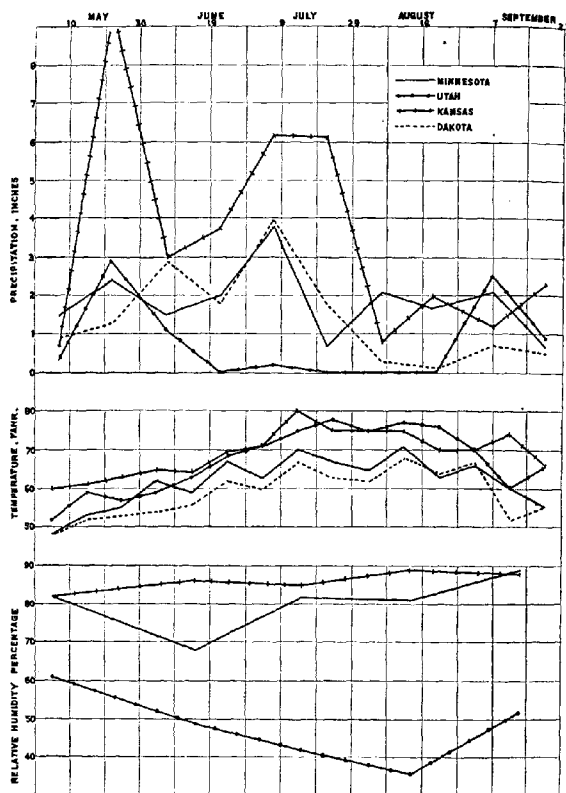


FIG. 3.—Curves showing the precipitation, temperature, and humidity relations at the various experiment stations during the growing season of 1915.

the plots grew nearly as fast as those at the other stations and gave a higher yield of dry cane at the end of the season than did the South Dakota plots, although the latter grew much taller. The Kansas plots grew the most rapidly.

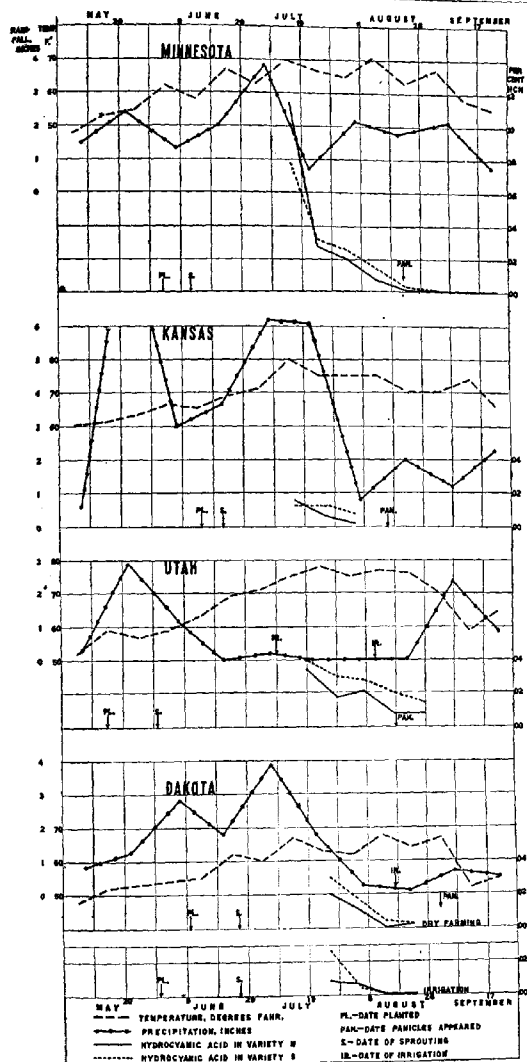


Fig. 4.—Curves showing the contemporary climatic conditions at the various plots, together with crop data and hydrocyanic-acid content.

Accompanying these various conditions were also widely differing amounts of hydrocyanic-acid glucosid. How the correlations between these two may be explained will depend upon the function assigned to the dhurrin in sorghum. Various uses have been attributed to glucosids in plants, as (a) a protection against bacteria and other enemies by means of the poison set free when some glucosids are hydrolyzed; (b) a reserve food material in the plant; (c) the inactive form of a stimulating hormone (2) set free when necessary by a glucosidase; (d) a harmless compound absorbing injurious products of metabolism; (e) an inactive storage of "respiratory pigments," and other uses.<sup>1</sup> Hydrocyanic acid itself is thought by some investigators to be a necessary intermediate product in protein formation (6, 8). As such, it is probably rather transitory in the plant, and seldom occurs free in any appreciable amount.

A discussion of each factor which might have any bearing on the cause of the variations in cyanid content, or throw any light on the function of dhurrin in sorghum, follows.

1. HUMIDITY.—It is hard to perceive how the relative humidity might have any direct bearing on the quantity of dhurrin produced. The humidity affects primarily the rate of transpiration, and this in turn might influence the rate of growth. The latter factor is considered in the next paragraph. The interesting thing to note in the humidity curves in figure 2 is the fact that the Utah curve shows a decrease during a period of decreasing precipitation, which is natural, but the Kansas and Minnesota curves show an increase during periods of decreasing precipitation. It is possible that this very low humidity in Utah caused a rate of transpiration too high for the best development of the plants, and their growth was retarded accordingly. When the humidity was lowest, in July and August, the plots received their two irrigations. Following these the growth was more rapid. If the humidity affects the amount of glucosid at all, it is by means of its effect on the nutrition and growth of the plant.

2. MOISTURE SUPPLY.—As mentioned above, there are among the four stations one having very high, one with very low, and two with medium rainfall. Two plots at the last-named stations were under irrigation and one under dry-farming methods of cultivation. In the data as a whole there is no evident correlation between the amount of the glucosid and the moisture supply for the five months. Arranging the stations in the order of their moisture supply, they are Kansas, Minnesota, South Dakota irrigation, South Dakota dry farming, and Utah; while arranged in the order of their cyanid content they are Minnesota, Utah, Kansas, and South Dakota dry farming the same, and South Dakota irrigation. However, by a closer examination of the curves for

<sup>1</sup>For a complete discussion of the function of glucosids in plants see Armstrong, E. F., *The Simple Carbohydrates and the Glucosids*. Ed. 2, p. 175-131. London, New York, 1912.

each plot, the following examples tending to show that high water supply is often accompanied by a low cyanid content are discernible: (1) The normal hydrocyanic-acid curve for sorghum during the first two-thirds of its growth is a smooth curve, with a steady decrease in the acid. The Utah curve is an exception to this. In this plot there was for several weeks very rapid transpiration of water, owing to low humidity; hence, the plants and soil were reduced nearly to the minimum water requirement. Shortly after the first irrigation the hydrocyanic acid is seen to be on a normal decline. Twenty days after this irrigation, however, the plots had become comparatively dry again, and the hydrocyanic acid shows a less decrease in variety S and an actual increase in variety N. The second irrigation was followed by another decline in hydrocyanic acid. By the latter part of August the need of water was once more felt, and the cyanid in variety N, at least, had ceased to decrease. (2) The curve for the South Dakota dry-farming plot also shows an abnormality in that the last part of it has an upward turn in the case of variety N. It is possible that this may be due to the smaller supply of moisture available at this time. (3) In the two South Dakota plots, both received the same amount of rain; one was irrigated once and the other, being cultivated by dry-farming methods, had a larger reserve supply of water. This would apparently give them about the same amount of water supply, except for the fact that the irrigation was a heavy one, and the heavy rains during May, June, and July disturbed the usual dry-farming condition of the soil. Assuming that the irrigated plots did have more water available, it will be seen that they also contained a less amount of hydrocyanic acid. (4) On analyzing some sorghum plants grown in pots in the greenhouse, they were found to contain no hydrocyanic acid. A few weeks later some larger plants from this same group, growing in drier soil, owing to lack of care in watering and to a larger demand made by the plants, were found to contain some of the acid. There appears, therefore, to be a relation between the supply of water and the amount of dhurrin present. This may be explained on the hormone theory. With a liberal supply of water, other things being equal, the plant's means for growth are adequate and it needs less glucosid. With a decreasing water supply, however, the plant may need the hormone stimulus for growth, and more glucosid is produced. Although, as shown by Briggs and Shantz (5), sorghum has a lower water requirement than most cultivated plants, it is no doubt affected by changes in the supply of moisture.

3. TEMPERATURE.—No correlation has been found between the content of dhurrin in sorghum and variations in temperature, at least for the range of temperatures which obtained during this experiment. The increase in hydrocyanic acid which sometimes occurs when plants are frosted may be due to disturbed enzym balance.

4. RATE OF GROWTH.—The Kansas and Utah plots present extremes in rate of growth and thriftiness of the sorghum plants; and they also present cases of relatively low and high hydrocyanic-acid content, respectively. The cane on the Minnesota plots grew more slowly than that from South Dakota, and it also contains a very much higher hydrocyanic-acid content. During the first four or five weeks on the Minnesota plots the plants grew very poorly, the weather being cold and damp. The plants were yellow and uneven in height, similar to those obtained from Utah. The samples from these two stations were by far the highest in hydrocyanic acid. In fact, the percentage in the first Minnesota sample, variety N (0.114 per cent), is the highest ever observed in the authors' experience with sorghum. In the Minnesota samples of 1914 those grown on the poorer sandy soil were the higher in cyanid. These examples, together with one furnished by Avery (3), show that some significant relation may exist between poor conditions of growth and high dhurrin content. In opposition to this, however, is the finding of Alway and Trumbull (1) that the yellowier plants in a field contained a smaller amount of the acid. Balfour (4) found more in plants infested with *Aphis sorghi* than in others not so affected. If these facts are now applied to the various theories mentioned above, as to the function of glucosids, some of the possibilities are as follows: (1) If this particular glucosid is a food storage, it is difficult to see how it could exist in largest quantities in the unhealthy, poorly nourished, slow-growing plants. (2) If the constituents of the glucosid act as stimulating hormones when set free by an enzyme, it is possible that when conditions of growth are poor more of the glucosid is produced. (3) If the glucosid is an absorber of harmful products of metabolism under disturbed metabolic conditions, an excess of hydrocyanic acid might be produced. Of these three the authors believe the second to be the most tenable for dhurrin, according to the available evidence on this question.

5. VARIETY.—The most striking phenomenon in this experiment is the fact that Variety S has consistently a greater amount of hydrocyanic acid than Variety N. That varietal difference is very important was brought out also in the 1914 experiments. In fact, the authors are confident that the most marked and constant differences in the hydrocyanic-acid content of various sorghum plants will be found to be due to variety rather than to external conditions. A comparative study of the glucosid content of all varieties of sorghum would be interesting and valuable.

6. DISTRIBUTION IN THE PLANT.—The foregoing discussion has been based on the dhurrin content of the whole plant. As is seen from Table II, the distribution of the glucosid between stalk and leaves in the different plots is variable. There is in every instance a more rapid decrease in the stalks than in the leaves, but the comparative rate of decrease varies. The Minnesota and Utah plots had the highest amount in the stalks and also had the slowest growth and the thinnest stalks. The Kansas and

the South Dakota plots, on the other hand, had little or no cyanid in the stalks and had the most rapid growth. The Kansas stalks were very heavy and succulent; they had developed very rapidly; and they contained no cyanid whatever. The significance of this is not clear.

7. DAILY VARIATION.—In order to compare the glucosid content in sorghum with Treub's findings (7, 8) that in *Pangium edule* there is a daily variation in glucosid content with a maximum about midday, some analyses were made at sunset and sunrise of succeeding days, with the results given in Table III.

TABLE III.—Variation in the glucosid content of sorghum at different parts of the day

Variety.	Part of plant.	Percentage of hydrocyanic acid.	
		Evening.	Morning.
Variety N.....	Leaves.....	0.015	0.012
Do.....	Stalks.....	.013	.014
Dakota Amber.....	Leaves.....	.020	.023
Do.....	Stalks.....	.023	.031

There seems to be no constant variation in sorghum between night and day. This lends support to the view that dhurrin is not a food storage.

Although other factors have important bearing on the growth and health of plants, those discussed above are the most readily measured and, hence, best used as bases for comparison between widely separated stations. It is realized that determinations of soil moisture at various times throughout the growing season would give a much more accurate idea of the available moisture than precipitation measurements. As regards soil, each plot was grown on soil which has produced good crops in the past and was cultivated according to the customary methods for sorghum at those stations. Since the 1914 experiments showed that soil is a minor factor in affecting the hydrocyanic-acid content of sorghum, the ignoring of this factor in the above comparisons is justified.

#### SUMMARY

Two varieties of sorghum, Southern Cane and Early Amber, were grown on plots in Minnesota, Utah, Kansas, and South Dakota under widely different climatic and cultural conditions. The amount of the glucosid dhurrin in each plot varied considerably. The following correlations relative to the amount of glucosid were found to exist.

(1) Unhealthy plants usually contain more hydrocyanic acid than healthy ones. The unhealthy condition may be due to malnutrition, to improper transpiration, to insect attack, or to other causes. It is possible that under such conditions the plant produces more glucosid for the sake of the stimulating hormones in it.

(2) The apparent effect of humidity and temperature on the amount of cyanid in sorghum is probably due to the indirect effect on the rate of growth.

(3) Adequate water supply is usually accompanied by low, and inadequate by high, hydrocyanic-acid content. This is probably due to the need of glucosid stimulation when the water supply becomes low.

(4) The character of the growth of the plant affects the distribution of dhurrin between leaves and stalks, there being a proportionately smaller amount in the thick, heavy stalks than in the slender ones.

(5) There is no consistent daily variation in the amount of dhurrin, which argues against the functioning of this glucosid as a food storage.

(6) Of the two varieties used in this experiment, the Southern Cane in every plot but one had a higher content of hydrocyanic acid than the Early Amber. Varietal difference is probably of more weight in determining the amount of hydrocyanic acid in sorghum than are the conditions of growth.

#### LITERATURE CITED

- (1) ALWAY, F. J., and TRUMBULL, R. S.  
1910. On the occurrence of prussic acid in sorghum and maize. *In* Nebr. Agr. Exp. Sta. 23d Ann. Rpt., 1909, p. 35-36.
- (2) ARMSTRONG, H. E., and ARMSTRONG, E. F.  
1911. The function of hormones in regulating metabolism. *In* Ann. Bot., v. 25, no. 98, p. 507-519.
- (3) AVERY, Samuel.  
1902. Laboratory notes on poison in sorghum. *In* Jour. Comp. Med. and Vet. Arch., v. 23, no. 11, p. 704-706.
- (4) BALFOUR, Andrew.  
1904. Cyanogenesis in sorghum vulgare. *In* 1st Rpt. Wellcome Research Lab. Gordon Mem. Col. Khartoum, [1903/04], p. 46-48.
- (5) BRIGGS, L. J., and SHANTZ, H. L.  
1914. Relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 1-63, pl. 1-7.
- (6) RAVENNA, Ciro, and ZAMORANI, Mario.  
1909. Nuove ricerche sulla funzione fisiologica dell'acido cianidrico nel Sorghum vulgare. *In* Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., v. 18, sem. 2, fasc. 8, p. 283-287.
- (7) TREUB, Melchior.  
1907. Notice sur "l'effet protecteur" assigné à l'acide cyanhydrique des plantes. *In* Ann. Jard. Bot. Buitenzorg, v. 21 (s. 2, v. 6), pt. 1, p. 107-114, pl. 3-4.
- (8) ———  
1909. Nouvelles recherches sur le rôle de l'acide cyanhydrique dans les plantes vertes. III. *In* Ann. Jard. Bot. Buitenzorg, v. 23 (s. 2, v. 8), pt. 1, p. 85-118, pl. 18-23 (col.).
- (9) VIEHOEVER, Arno, and JOHNS, C. O.  
1915. On the determination of small quantities of hydrocyanic acid. *In* Jour. Amer. Chem. Soc., v. 37, no. 3, p. 601-607.
- (10) WILLAMAN, J. J., and WEST, R. M.  
1915. Notes on the hydrocyanic-acid content of sorghum. *In* Jour. Agr. Research, v. 4, no. 2, p. 179-185, 2 fig. Literature cited, p. 185.

## EGG AND MANNER OF OVIPOSITION OF LYCTUS PLANICOLLIS<sup>1</sup>

By THOMAS E. SNYDER,

Assistant in Forest Entomology, Bureau of Entomology

### HISTORICAL SUMMARY

The so-called "powder-post" injury to seasoned wood products is widely distributed over the world. Of the various beetles causing this type of injury, species of the genus *Lyctus* Fab. are by far the most important. While these beetles and their damage have an extensive literature, the place and manner of oviposition have remained obscure. Heeger (3),<sup>2</sup> in 1853, described and figured the egg, larva, and pupa of a beetle attributed to a European species, *Lyctus pubescens* Panzer. Dugès (1),<sup>3</sup> in 1883, described and figured the larva, pupa, and adult of *L. planicollis* Le Conte (?), proving that Heeger was in error in ascribing the larva he figured to the genus *Lyctus*. Xamheu (7), in 1898, described the egg and manner of oviposition of *L. linearis* Goeze (*canaliculatus* Fab.). Recently the eggs of the native species, *L. planicollis* of the southern United States, have been found by the writer. This egg is very unlike that described and figured by Heeger as the egg of *L. pubescens*, and it differs from the egg of *L. canaliculatus* as described by Xamheu, being of a most unusual type for Coleoptera.

The following brief notes on the mating and oviposition of the southern species (*L. planicollis* Le Conte) were made on material being reared either at Washington, D. C., or at Falls Church, Va., in buildings kept dry and at a temperature above freezing.

### LIFE CYCLE

#### MATING

The beetle passes the winter in the larval stage, but in cold weather the larvæ are more or less dormant and infested stock may consequently pass unnoticed. Mating takes place and the eggs are deposited soon after the adult beetles emerge from the wood in the spring. At Washington, D. C., and Falls Church, Va., the first adults emerged from infested wood in rearing cages during the last part of February and first part of March,

<sup>1</sup> The specimens on which this paper is based were identified by Mr. W. S. Fisher, Specialist on Forest Coleoptera, of the Branch of Forest Insect Investigations, Bureau of Entomology.

<sup>2</sup> Reference is made by number to "Literature cited," p. 276.

<sup>3</sup> According to Dugès, the material on which his paper was based had been determined by two different authorities as *Lyctus planicollis* Le Conte (of southern U. S.) and *carbonarius* Walck. (of Mexico and Florida). Dugès refers to the species as *planicollis* in the title and *carbonarius* on the plate. Hopkins (5, p. 134) states that *L. carbonarius* is evidently distinct from *L. planicollis*, and therefore Dugès's specimens are *L. carbonarius*.



in 1914 and 1915. At Baltimore, Md., adults of this species emerged from an infested oak table in a heated building as early as January 12, 1916. General emergence at Falls Church, Va., however, did not begin until about the middle of April, 1914 and 1915. The period of maximum activity is from the last of April to the first part of June. The last adults emerged during the first part of July. Mating occurred commonly during May, in 1915.

#### OVIPOSITION

Oviposition began a few days after mating and was observed to take place principally during the middle of May, in 1915. On May 24, 1915, many beetles were observed on radial sections of wood with their ovipositors deeply inserted into the open ends of pores or large longitudinal vessels in the wood, but the first eggs were not found till June 1, 1915.

The beetles seem to prefer to oviposit on those sections of seasoned sapwood where the open ends of pores are most numerous. These pores are especially prominent in "ring-porous" woods such as hickory, ash, and oak, which are also the species most subject to attack by *Lyctus* beetles. No eggs were observed on the surface of the wood, but all that were found were in these pores.

The females remain for several minutes with the ovipositor in the pore, and the process is repeated at several places. The female usually assumes a position in which the body is parallel to the pore and the ovipositor is either curved down and bent forward into the pore underneath the body or projected directly into the open end of the pore. However, the ovipositor, which is long and flexible and reaches from the end of the body to the thorax when extended forward, can be projected in any direction. At the extremity of the ovipositor are two laterally placed palpi. In the process of inserting the ovipositor into the pores, there is a considerable preliminary period of thorough examination with these palpi of all parts of the pore before an egg is laid. Two or more eggs are usually laid near together in each pore utilized. Each female deposits eggs in several pores.

#### THE EGG

The egg (Pl. XXVIII, fig. 1) is cylindrical, rounded at the ends, and has a slender strand or process attached to the cephalic pole. It is whitish in color, somewhat shiny, 1 mm. in length with the strand attachment, 0.75 mm. in length without this process, and 0.175 mm. in width. This process or strand is somewhat similar to that of the eggs of certain parasitic Hymenoptera—that is, parasites of the cotton boll weevil in the families Eurytomidae and Encyrtidae (6, p. 49–51, pl. 2), but this is the only instance known to the writer of such a process on the eggs of Coleoptera. The egg has a granular appearance (Pl. XXVIII, fig. 2), and at the end which terminates in the process there is an area marked with parallel, longitudinal striæ (Pl. XXVIII, fig. 4). The egg of *L. linearis*

(*canaliculatus*), as described by Xamheu, is very different from the egg of *L. planicollis*, since no mention is made by Xamheu of either the strand attachment or the area of longitudinal striae, which are unusual characters in the egg of a beetle.

The end with the process (the cephalic pole) leaves the ovipositor last,<sup>1</sup> and this strand may possibly be attached by the ovipositor to the pore contents. The larva does not occupy much more than half the length of the egg (Pl. XXVIII, fig. 3). In hatching, the larva backs out of the egg. The eggs are easily broken, and it is probably due to this fragility and the fact that they are inserted far into the pores that the eggs of *Lyctus* beetles have apparently not been previously observed with absolute certainty of their identity.

#### SEASONAL HISTORY

Egg laying takes place principally during the middle of May. Recently hatched larvæ were first observed on June 1, 1915. The period of incubation is probably, at most, 10 days. The winter is passed in the larval stage. General pupation occurs about the first of April; the pupal cell (Pl. XXX) is excavated near the surface of the wood, and to this cell the larvæ retreat after cutting a transverse burrow nearly to the surface for the exit of the adults. General emergence of the adults takes place during May. Under normal conditions of the natural habitat of this species (in the Gulf and South Atlantic States) activity probably occurs earlier in the season.

There is apparently only one generation annually. But the combined work of the many larvæ of successive broods and generations burrowing through the wood results in the complete destruction of the interior and the conversion of the wood into fine powder—that is, "powder-posted" wood (Pl. XXIX, XXX, and XXXI).

#### CONCLUSIONS

Injury by "powder-post" beetles to unfinished seasoned wood products can be prevented by simply adapting a system of inspection, classification, and methods of disposal of stock to facts in the seasonal history of the insects, as has been recommended for many years by Hopkins (4, p. 6), *Forest Entomologist*. Such methods have been adopted by several large manufacturing companies with marked success.

In the case of finished wood products it may often be practicable to treat the wood with substances to prevent attack. Creosotes are effective preventives, but they stain the wood; hence, where they can not be used, in the light of the discovery of the place and manner of the laying of the eggs, any substance that will close the pores will prevent oviposition in wood not previously infested. In wood from which beetles have

<sup>1</sup> This is according to the law of orientation of Haliez (2).

emerged, however, eggs might be laid within the exit holes. Paraffin wax, varnish, or linseed oil effectively closes the pores of wood. Wood that has been seasoned less than 8 to 10 months will not be attacked by Lyctus beetles. In applying chemical preventives, only sapwood that has been seasoned for 8 to 10 months and longer should be treated. Judging from facts in the seasonal history of this species, preventives should be applied before March 1.

## LITERATURE CITED

- (1) DUGÈS, E.  
1883. Métamorphoses du *Lyctus planicollis* Le Conte. *In* Ann. Soc. Ent. Belg., t. 27, p. 54-58, 1 pl.
- (2) HALLEZ, Paul.  
1886. Loi de l'orientation de l'embryon chez les insectes. *In* Compt. Rend. Acad. Sci. [Paris], t. 103, p. 606-608.
- (3) HERGER, Ernst.  
1853. Beiträge zur Naturgeschichte der Insecten. *In* Sitzber. K. Akad. Wiss. [Vienna], Math. Naturw. Kl., Bd. 11, Heft 5, p. 927-942, 6 pl. Also reprinted.
- (4) HOPKINS, A. D.  
1910. Insects injurious to forest products. U. S. Dept. Agr. Bur. Ent. Circ. 128, 9 p.
- (5) KRAUS, E. J., and HOPKINS, A. D.  
1911. A revision of the powder-post beetles of the family Lyctidae of the United States and Europe. Pt. III. U. S. Dept. Agr. Bur. Ent. Tech. Ser. 20, p. 111-138.
- (6) PIERCE, W. D., CUSHMAN, R. A., and HOOD, C. E.  
1912. The insect enemies of the cotton boll weevil. U. S. Dept. Agr. Bur. Ent. Bul. 100, 99 p., 3 pl., 26 fig.
- (7) XAMBEU, V.  
1898. Mœurs et métamorphoses du *Lyctus canaliculatus* Fabricius. *In* Bul. Soc. Sci. Nat. Ouest France, t. 8, p. 69-72.



PLATE XXVIII

*Lyctus planicollis:*

Fig. 1.—Outline of the egg, showing strand attachment.

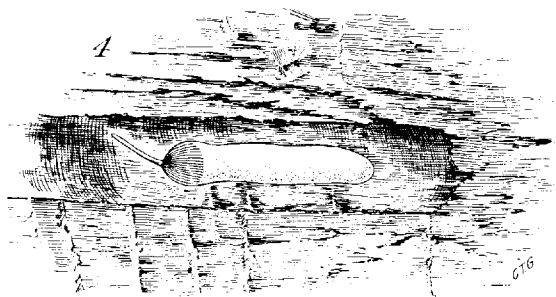
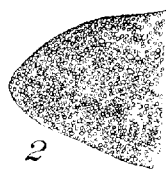
Fig. 2.—Greatly enlarged view of end of egg, showing granular appearance.

Fig. 3.—Larva within egg, ready to hatch. Drawn by Miss M. Carmody.

Fig. 4.—Sketch of egg in pore of wood on radial section of green-ash (*Fraxinus lanceolata*) ladder-rung stock, showing longitudinal striæ; pore opened to show egg.  
Drawn by C. T. Greene.

*Lycus planicollis*

PLATE XXVIII



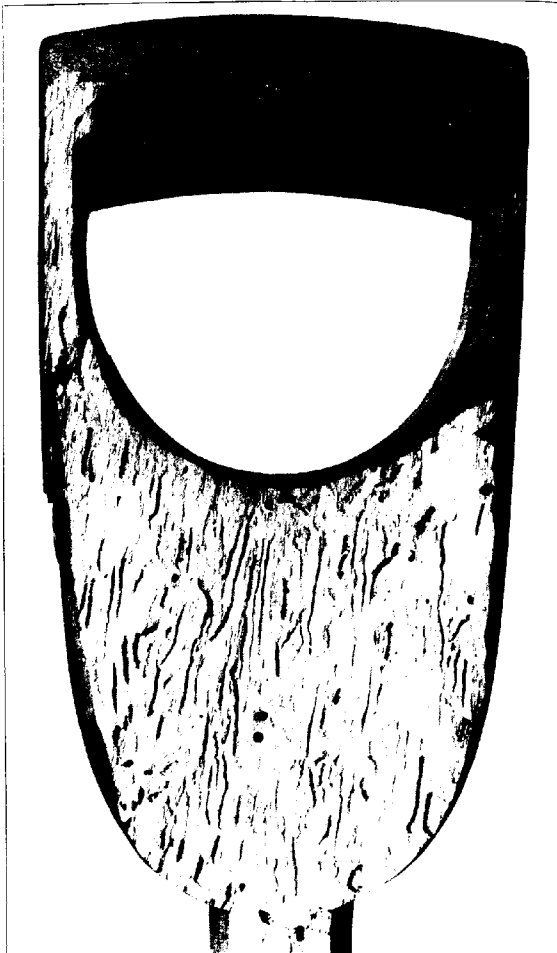


PLATE XXIX

*Lyctus planicollis:*

Larval burrows in an ash shovel handle. Handle planed to show the work of the larvæ. Photographed by H. B. Kirk.



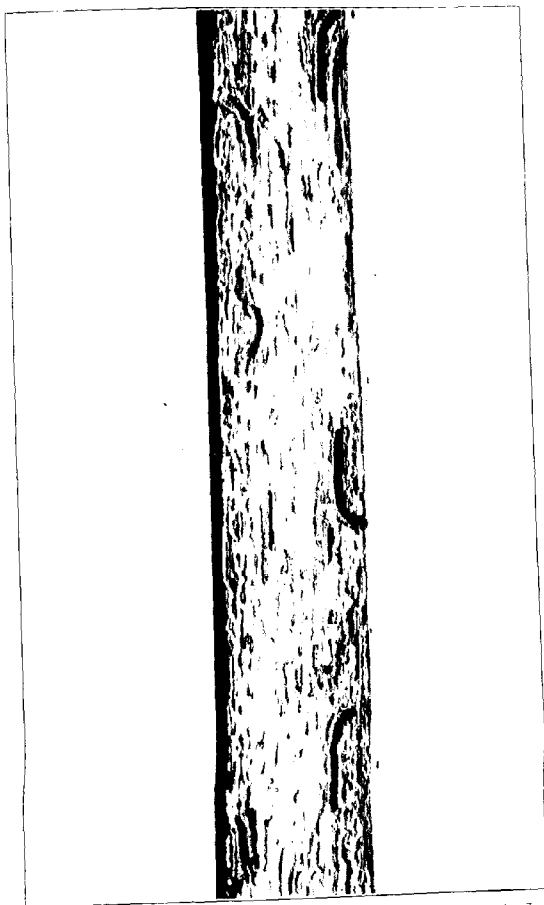
PLATE XXX

*Lyctus planicollis:*

Pupal cells in "powder-posted" white-ash shovel handle. Photographed by  
H. B. Kirk.

*Lyctus planicollis*

PLATE XXX

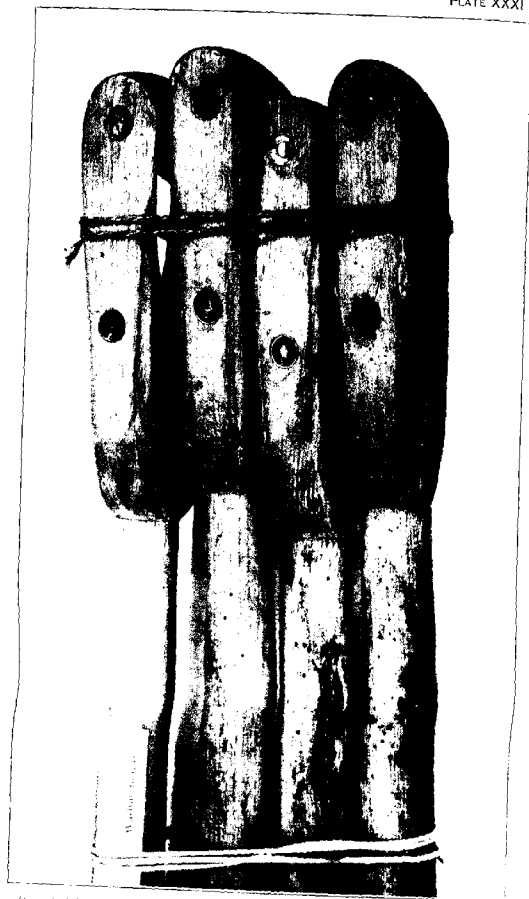


*Journal of Agricultural Research*

V. L.V., No. 7

*Lystus planicollis*

PLATE XXXI



*From the American Museum of Natural History*

Vol. VI, No. 7

PLATE XXXI

*Lycus planicollis*:

Exit holes of adults in ash shovel handles. Photographed by H. B. Kirk.